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1
AN 134:233815 CA
TI Physical perturbation for fluorescent characterization of microorganism particles
AU Bronk, Burt V.; Shoaibi, Azadeh; Nudelman, Raphael; Akinyemi, Agnes N.
SO Proceedings of SPIE-The International Society for Optical Engineering (2000), 4036(Chemical and Biological Sensing), 169-180.

2
AN 131:141627 CA
TI Fluorescence of dipicolinic acid as a possible component of the observed UV emission spectra of bacterial spores
AU Nudelman, Raphael; Feay, Nicole; Hirsch, Mathew; Efrima, Shlomo; Bronk, Burt
SO Proceedings of SPIE-The International Society for Optical Engineering (1999), 3533(Air Monitoring and Detection of Chemical and Biological Agents), 190-195

3
AN 135:192324 CA
TI Ultraviolet fluorescence imaging applications
AU Hill, Ralph H., Jr.; Angell, Peter
SO AT-PROCESS (2000), 5(3,4), 108-114

4
AN 123:51436 CA
TI Spectroscopic properties of tryptophan and bacteria
AU Tang, G. C.; Yang, Y. L.; Huang, Z. Z.; Hua, W.; Zhou, F.; Cosloy, S.; Alfano, R. R.
SO Proceedings of SPIE-The International Society for Optical Engineering (1995), 2387, 169-72

5
AN 120:265052 CA
TI Online, non-destructive biomass determination of bacterial biofilms by fluorometry
AU Angell, Peter; Arrage, Andrew A.; Mittelman, Marc W.; White, David C.
SO Journal of Microbiological Methods (1993), 18(4), 317-27

Fluorescence of Dipicolinic Acid as a Possible Component of the Observed UV Emission Spectra of Bacterial Spores

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Abstract

Dipicolinic acid (DPA) and the Ca^{2+} complex of DPA (CaDPA) are well-known and are major chemical components of bacterial spores. DPA's native fluorescence is very weak and is thought to be completely masked by the fluorescence of tryptophan when this compound is present. Thus fluorescence related to DPA in spores is assumed by many authors to be completely absent. We show that the fluorescence of CaDPA is substantial for excitation between about 290 nm and 310 nm with emission peaking near 400 nm. This emission is at the long wavelength tail for emission from tryptophan. We examine whether the emission of CaDPA could contribute to the total emission spectrum when bacterial spores are present in an aerosol, for excitation wavelengths in the neighborhood of 310 nm. In this report we present measurements of fluorescence excitation and emission for CaDPA and compare them with that of DPA and tryptophan.

Keywords: dipicolinic acid, bacterial spores, fluorescence.

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1. Introduction

A number of reports have been published recently describing instrumentation utilizing fluorescence emission stimulated by laser excitation in the ultraviolet (UV) region, to detect and partially characterize the biological content of an aerosol.[1-4]

The biological component of an unknown aerosol may include both bacteria and bacterial spores. The ultraviolet (UV) stimulated emission from various bacteria has sometimes been reported to show sufficient variation from species to species to give at least a partial characterization of what bacteria are present in a liquid suspension.[5,6] On the other hand, a more recent report showed that well-washed bacteria and bacterial spores of several different species each gave exactly the same spectrum for excitation within the range 250 to 300 nm.[4] except perhaps for the amount of emission per particle.

The UV stimulated fluorescence from both bacteria and bacterial spores has been attributed mainly to tryptophan.[7] Fluorescence from the other two common aromatic amino acids has yet to be demonstrated as a component of the emission from whole microorganisms. Dipicolinic acid (2,6 pyridinedicarboxylic acid) is a major component of bacterial spores, constituting 5 to 15% of the spore by weight compared to about , 5% for tryptophan.[8] Comparing the Raman spectra for *Bacillus cereus* spores with measured spectra of DPA or CaDPA, Shibata *et. al.*[9] indicated that the DPA in the spores is almost all complexed with calcium.

Pure DPA absorbs in the UV, but has negligible fluorescence for UV excitation,[10] with a weak emission at 333 nm for excitation at 277.8 nm.[11] Barela and Sherry reported[10] that DPA complexed with terbium fluoresces strongly. This effect has recently been shown to be useful for detecting bacterial spores in the presence of contaminants.[12,13] We previously reported preliminary indications,[2] that a solution of DPA and $Ca(OH)_2$ does fluoresce for excitation in the 285-315 nm range with a peak at ~ 400 nm. In the present report we present a controlled and semi-quantitative determination of the fluorescence of CaDPA under various conditions.

2. Experimental

All chemicals used were purchased from Aldrich. Stock solutions at high concentrations (typically 10 mM) were prepared. Dilutions into distilled deionized water were done on the day of the experiment.

Instrumentation used included a Milton Roy 601 Spectronic spectrometer for absorption and a Spex Fuorolog-2 Spectrofluorometer. The Fluorolog is equipped with double grating excitation and emission spectrometers. All readings taken are automatically compared with a built-in rhodamine B standard, to compensate for lamp intensity changes and changes in sensitivity across the excitation band.

Concentrations of DPA for fluorescence were in the range of 10-100 mM and Ca^{++} concentrations either equaled or exceeded this. Spectra were taken with the solutions at pH in the range 7.5 to 9.0. The solutions were kept covered to prevent them from becoming acidified by absorption of CO_2. The preparation of Na_2DPA and CaDPA was done as in Ghiamati *et al.*[14] with NaOH in excess of DPA. The solutions were monitored to insure they were maintained in the desired pH range.

3. Results

Typical absorption spectra for DPA, Na_2DPA, CaDPA and L-tryptophan are shown in Figure 1. The double peak verifying the presence of CaDPA[15] is clearly present.

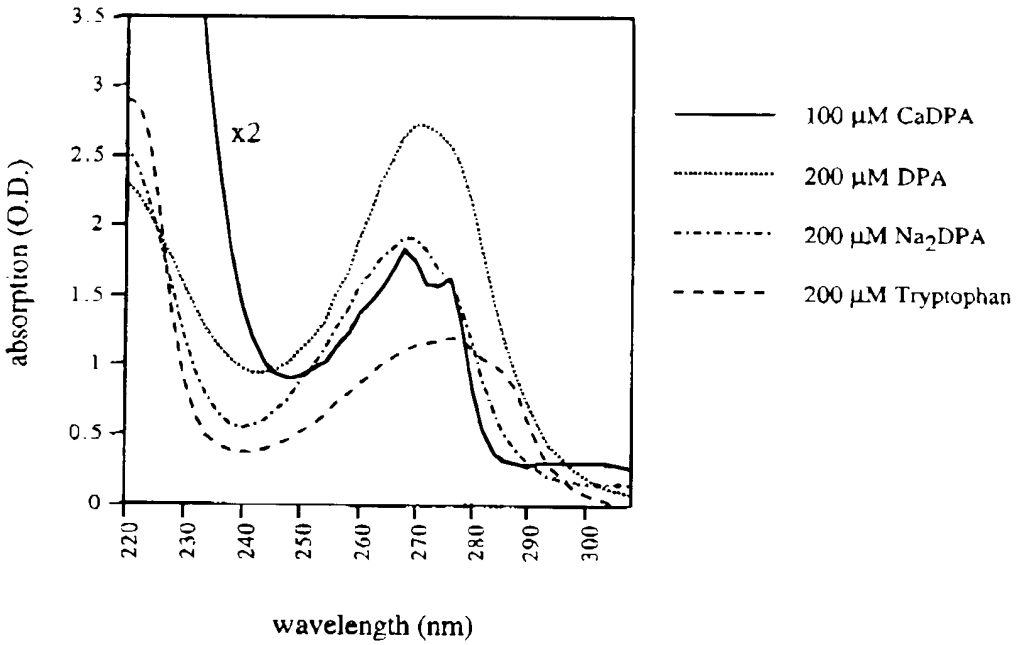

Fig 1. Absorption spectra of CaDPA, DPA, Na_2DPA and L-tryptophan in aqueous solutions at pH 8.5.

In Figure 2 we see the emission spectra for the same four compounds with excitation at 305 nm. We note that under these circumstances there is a quite pronounced maximum peaking at about 402 nm for CaDPA. Tryptophan also excited at 305 nm has its emission peak at 360 nm, but still has a considerable emission at 402 nm for this excitation wavelength, so that the molar concentration for the curve shown is 10 fold lower than that of the CaDPA. The relative intensities should be considered with caution as we noticed variations with time under illumination.

Figure 3 shows that when exciting at 295 nm the fluorescence emission of the CaDPA is completely swamped by that of the tryptophan (note the widely different concentrations).

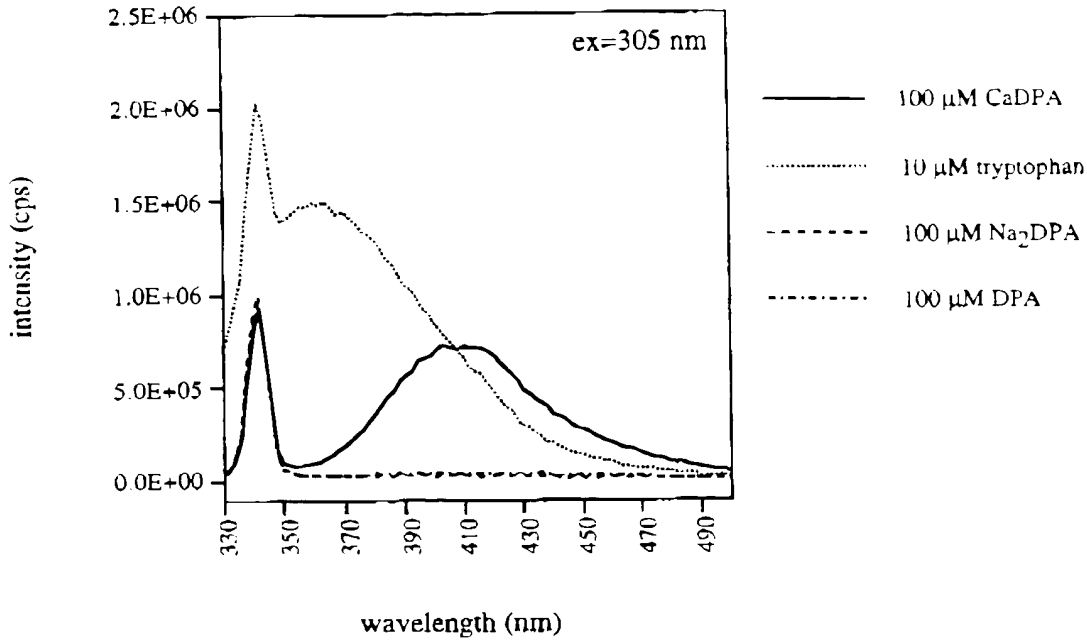

Fig. 2. Emission spectra of CaDPA , L-tryptophan, Na_2DPA andDPA in aqueous solutions at pH 8.5, when excited at 305 nm.

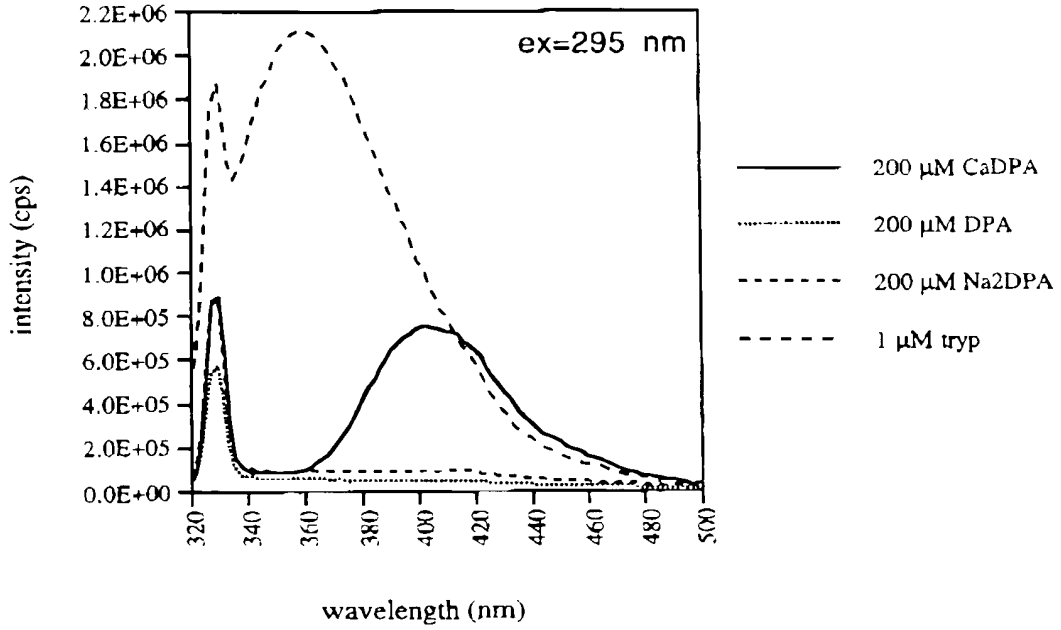

Fig. 3. Emission spectra of CaDPA, DPA, Na_2DPA and L-tryptophan in aqueous solutions at pH 8.5, when excited at 295 nm.

In Figure 4 we present an excitation spectrum for CaDPA and L-tryptophan with emission at 402 nm. The quantum efficiency of CaDPA appears to be considerably higher in the neighborhood of 300 nm rather than where its absorption maxima occur at about 268 to 277 nm.

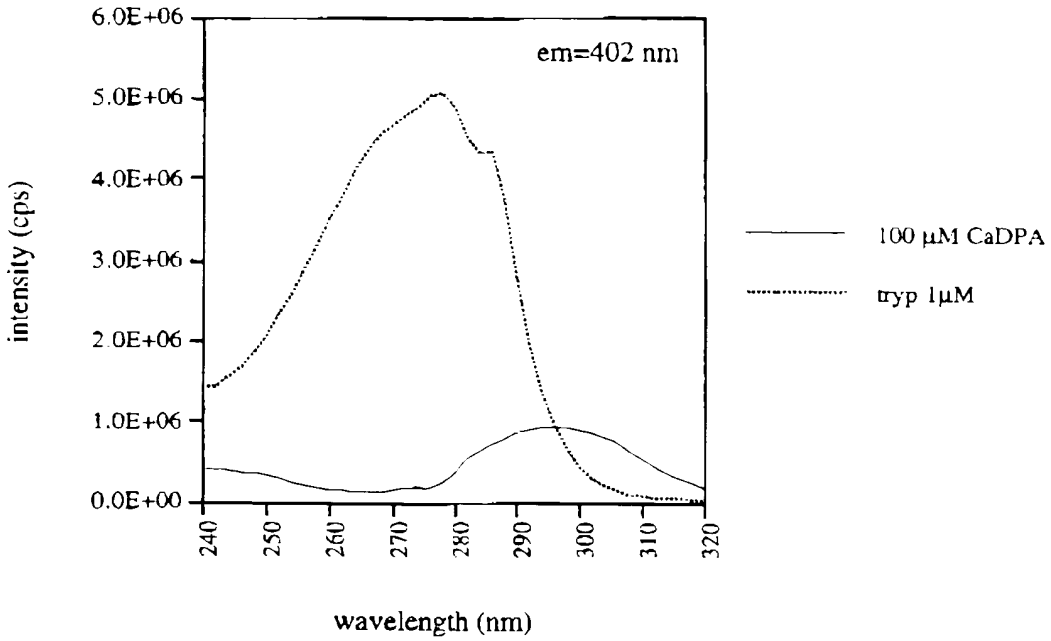

Fig. 4. Excitation spectrum of CaDPA and L-tryptophan in aqueous solutions at pH 8.5.

4. Discussion

CaDPA, when formed with doubly ionized DPA, clearly fluoresces well above the level of its constituents, but with a substantially lower fluorescence yield than tryptophan for excitations below 300 nm. At an excitation wavelength of 305 nm, we find that the fluorescence emission at ~402 nm of the two compounds is significant. In tryptophan's natural environment in the spore the molecules in the vicinity of tryptophan could affect (decrease?) the fluorescence emission compared to that measured *in vitro*. Thus if the results presented here can be extrapolated to spores, then at excitations around 305 nm, the CaDPA may account for some of the total emission spectrum.

Extreme care should be exercised when working with buffer solutions due to solubility, interaction and background interferences. The photophysics of DPA and its calcium complex are intricate and remain to be elucidated.

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